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TECHNICAL MANUSCRIPT 445

USE OF PLASTIC T-30 TISSUE CULTURE FLASKS UNDER TIME-LAPSE PHASE-CONTRAST MICROSCOPY

Louis E. Schneider
Robert R. Rosato

APRIL 1968

DEPARTMENT OF THE ARMY
Fort Detrick
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April 1968

USE OF PLASTIC T-30 TISSUE CULTURE FLASKS UNDER TIME-LAPSE PHASE-CONTRAST MICROSCOPY

ABSTRACT

With the long-working-distance phase-contrast condensers now available, it is possible to use plastic T-30 tissue culture flasks in phase-contrast time-lapse cinephotomicrographic studies. Data and photographs obtained with such a system are presented, and the advantages of using plastic flasks are discussed.

A continuing need exists to study both the gross effect of viral infection upon mammalian cells and the precise generation times of uninfected mammalian cells. This dual need has been filled by the use of Sage cinephotomicrographic equipment* that incorporates a Bolex 16-mm motion-picture camera** and a Zeiss-Plankton inverted microscope supplied with a Zeiss Model I-S Zernicke-type phase-contrast condenser. This particular condenser was chosen for its long working distance, which permits the phase-contrast illumination of plastic T-30 tissue culture flasks*** when a 10X/0.22 Zeiss phase-contrast objective is used. Some data obtained with this system are presented in this study, which further considers both the advantages and difficulties encountered in use of plastic T-30 tissue culture flasks.

Living material studied under phase-contrast illumination reveals structural details otherwise visible only in killed and stained preparations. The ability to use phase-contrast illumination in conjunction with plastic T 30 tissue culture flasks is desirable for (i) its convenience, (ii) the availability of reliably clean, optical-quality growth surfaces, and most important, (iii) the retention of growth conditions identical with those of the normal laboratory propagation of monolayer cultures. Thus, data obtained can be directly related to parallel cultures carried in plastic T-30 flasks. Less valid comparative

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* Sage Instruments, Inc., White Plains, New York 10601.
** Model H-16M, Paillard S.A., Ste-Croix, Switzerland.
*** Falcon Plastics, Los Angeles, California 90045.

relationships can be drawn if the usual perfusion chamber, with its glass cover slips and sealing gasket, is used because of (i) the difference in the growth surfaces, both in cleanliness and in composition, and (ii) the absence of the prominent gas phase, which is characteristic of the normally propagated monolayer cultures.

Perfusion chambers are still needed for studies requiring the greatest resolution. Most microscope objectives are designed for use with glass cover slips that are either 0.17 or 0.18 mm thick (the former value is used by Continental manufacturers, the latter by English and American manufacturers).[1] A recent study[2] describes the incorporation of such cover slips into a plastic* Cooper dish,[3] which is then sealed at the edges. This system permits observation under high-resolution objectives and also retains a considerable gas phase.

Because high resolution was not required in these studies, the considerable thickness (0.9 mm) of the floor in the plastic tissue culture flask was not objectionable. Figure 1 shows an L-cell monolayer maintained in a plastic T-30 culture flask containing 5 ml of tissue culture medium with 10% bovine serum.** The cells were held under phase-contrast illumination and photographed on a 16-mm motion picture film*** by means of electronic flash.**** The cells were photographed under a 10X phase-contrast objective and a 5X planar eyepiece and at the 2X magnification of a Zeiss Optovar attachment.

The Optovar is a turret-type magnifier located between the microscope objective and eyepiece. In addition to the four magnification powers, 1X, 1.25X, 1.6X, and 2X, the Optovar also contains a magnifier for aligning the phase rings. Using this attachment, one can easily change the magnification of the field or check the alignment of the phase-contrast illumination during the course of a time-lapse experiment.

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* Falcon Plastics, Los Angeles, California 90045.

** Rosensteel, J.F.; Jordan, W.C.; Daniels, W.F. Growth potential of a variation of Eagle's minimal essential media for spinner culture. Unpublished data.

*** Kodak Ektachrome MS, daylight.

**** Flashtube FT-230, General Electric Co.

Figure 1. L-Cells in a Plastic T-30 Tissue Culture Flask, Phase-Contrast Illumination, Time-Lapse Cinephotomicrography. 200X.

Time-lapse studies for the determination of precise generation times were performed on the greatest possible field of view, achieved by using the Optovar at 1X, with a 10X phase-contrast objective and a 5X planar eyepiece. At this magnification and at a time-lapse speed of one frame every 2 minutes, the contrast proved adequate for the differentiation of cellular cleavage, and even cellular death, between two successive motion picture frames. Figure 2 is a diagram of the growth and progeny of a single mouse L cell maintained and photographed under the conditions just described. The progeny of this cell at 80.2 hours of growth are shown in Figure 3. Two of the cells are dead (the round cells), and two are still alive (the cells with pseudopods). The pseudopods are indicative of the cellular mobility that necessitated a single recentration of the cells to keep them all in view.

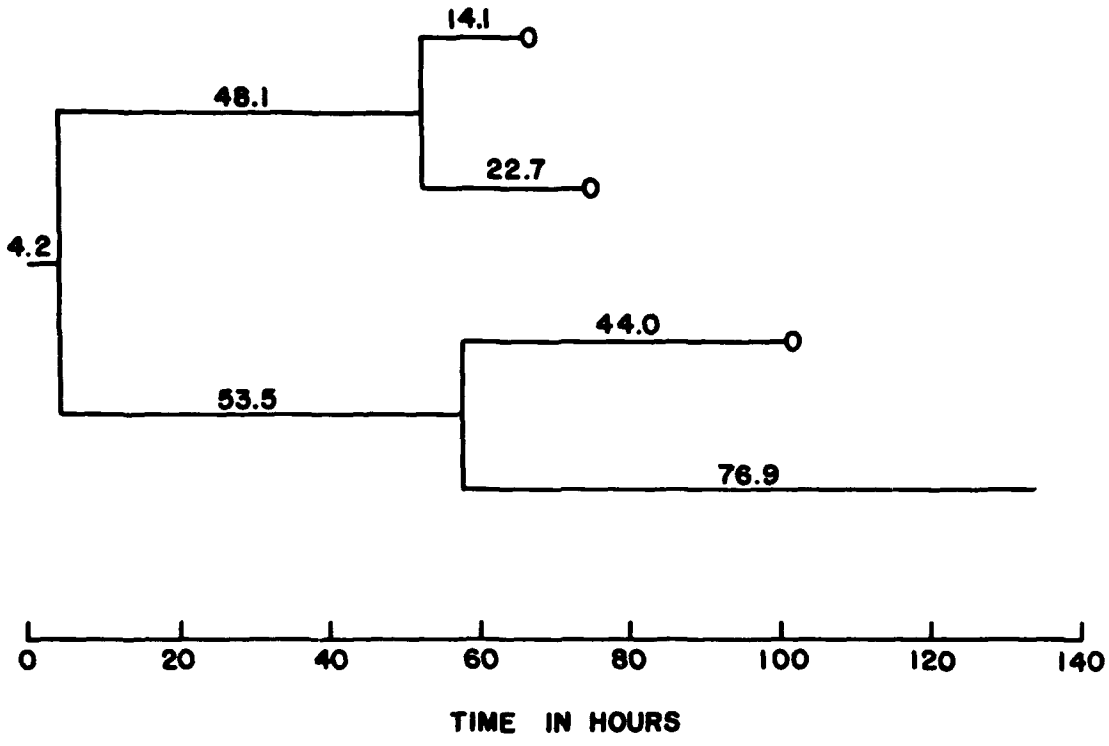

Figure 2. Growth and Progeny of a Single L-Cell from a Culture in a Plastic T-30 Tissue Culture Flask, Phase-Contrast Illumination, Time-Lapse Cinephotomicrography. O = death. Vertical line = cellular cleavage. Horizontal line = elapsed time in hours between cellular cleavages.

Figure 3. Progeny at 80.2 Hours of the Single L-Cell Described in Figure 2. 100X.

An unforeseen difficulty was encountered in the use of plastic T-30 flasks. Although the cellular monolayer was in focus at the start of an experient, the cells were considerably out of focus less than a day later. After the microscope itself was eliminated as the source of the difficulty, we discovered that the floor and the ceiling of the flasks were not rigid enough to prevent their minute bulging because of gaseous expansion within the sealed flask. Because preliminary temperature equilibration of the flasks and the cultures did not solve the difficulty, we suspected that the cells themselves might be modifying the sealed atmosphere. For this reason, the following simple mechanical expedient was devised. A 1/8-inch-diameter hole was drilled into the center of the tops of spare Bakelite caps without permitting the drill to pierce the white rubber lining. The holes were filled flush with a silicone-rubber compound,* and the caps were conveniently autoclaved in a glass petri dish. Such a modified cap was aseptically substituted for the regular cap of a monolayer-containing plastic T-30 flask and tightly screwed down. A sterile, disposable hypodermic needle (19-gauge, 1.5-inch) containing sterile, nonabsorbent cotton in the hub was then aseptically pushed through the rubber port in the cap.

The preparation of separate, sterile caps and needles was the most suitable method when infectious material was to be studied. The cap with a port was added in a biological safety cabinet, and the cap was sealed to the neck of the flask with plastic tape. The outside of the flask was decontaminated as it was passed from the safety cabinet to a bench top, where the needle was inserted. When noninfectious material was to be studied, the drilled cap and needle were assembled as a single unit prior to sterilization.

During the preparation of the monolayers, the flasks were carefully examined to see that medium was not escaping through an improperly sealed seam. Leaky flasks were reported by Petursson and Fogh,[4] and we also discovered some. As an additional precaution, particularly when infectious material is to be studied, a bead of silicone-rubber compound can be run all along the seam of the flask.

The inoculating needle inserted through the cap served as an adequate vent for releasing the pressure from the flask without allowing a loss of carbon dioxide sufficient to change the pH of the medium. Flasks vented in this fashion have been used in 5-day time-lapse experiments involving as many as 100,000 cells per ml (500,000 cells per flask). At the end of each experiment the cells were viable (as evidenced by recent division) and still in focus, and the pH of the medium was identical to that of an unvented control flask.

* Lab RTV, Dow Corning Corp., Midland, Michigan 48640.

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14. Key Words

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*Flask
*Cinephotomicrography
*Phase microscope

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